

I claim:

- ✓ Sub 8a7
1. A method for purifying plasmid DNA from a mixture of same containing at least one host cell impurity comprising the following steps:
 - (a) forming a solution by adding sufficient salt to said mixture to allow selective binding of said at least one host cell impurity to a hydrophobic interaction media;
 - (b) contacting said solution containing plasmid DNA with said hydrophobic interaction media under conditions that said at least one impurity binds to the hydrophobic interaction media to form a complex; and
 - (c) collecting unbound plasmid DNA from said complex and hydrophobic interaction media;

wherein said method is conducted in the absence of solvents, detergents, glycols, hexamine cobalt, spermidine, and polyvinylpyrrolidone.

2. The method of claim 1 wherein the at least one impurity is selected from the group consisting of RNA, endotoxin, chromosomal DNA and protein.

3. The method for claim 1 wherein the at least one impurity is an endotoxin.

00573507 053600

4. The method of claim 1 wherein the salt comprises an anion or cation selected from the group consisting of acetate, phosphate, carbonate, SO_4^{2-} , Cl^- , Br^- , NO_3^- , Mg^{2+} , Li^+ , Na^+ , K^+ and NH_4^+ .

Sub 8
5. The method of claim 4 wherein the salt is ammonium sulfate in a concentration range of 2M to 4M.

6. The method of claim 5 wherein ammonium sulfate is present at a concentration of about 2M.

Sub 9
7. The method of claim 1 wherein the solution comprises sodium salts in a concentration range of 2M to 4M.

8. The method of claim 7 wherein the sodium salt is sodium chloride.

9. The method of claim 8 wherein the sodium salt is sodium chloride in a concentration of about 2M.

10. The method of claim 1 wherein the pH of the solution has a range of about 6.8 to about 7.4.

11. The method of claim 1 wherein the pH of the solution is about

7.4.

12. The method of claim 1 wherein the hydrophobic interaction media comprises a chromatography support with pendent hydrophobic groups.

13. The method of claim 12 wherein said pendent groups are selected from the group consisting of C_3 to C_{10} alkyl groups and mixtures thereof.

00550 05500
Sub a10
14. The method of claims 12 wherein the hydrophobic interaction media are selected from the group consisting of a methacrylate polymer or copolymer backbone bound to a least one of a propyl, butyl, hexyl, octyl, nonyl or decyl ligand.

Sub B3)
15. The method of claim 14 wherein the media is at least one of a methacrylate ethylene glycol copolymer backbone or a cross-linked agarose backbone.

Sub a11
16. The method of claim 12 wherein the resin is in the form of bead in the size range of 15 to 100 μm .

✓ 17. A method of separating supercoiled plasmid DNA from a mixture of supercoiled plasmid DNA and relaxed plasmid DNA and, optionally, at least one host cell impurity comprising the following steps:

9119
www
(a) forming a solution by adding a salt to the mixture of supercoiled plasmid DNA and relaxed plasmid DNA and, when present, said at least one host cell impurity;

00350-05300
(b) contacting the solution with a hydrophobic interaction media under a first conditions where both the supercoiled plasmid DNA and relaxed plasmid DNA bind to the hydrophobic interaction media to form a bound first mixture;

(c) altering the first conditions surrounding the bound first mixture to a second conditions to remove relaxed plasmid DNA from bound first mixture to form separate components containing a second bound mixture and relaxed plasmid DNA; and

(d) modifying the second conditions surrounding the said bound second mixture to a third conditions to remove supercoiled plasmid DNA from said second bound mixture to form separate components containing hydrophobic interaction media and supercoiled plasmid DNA.

18. The method of claim 17 wherein the at least one host cell impurity is selected from the group consisting of RNA, endotoxin, chromosomal DNA and protein.

19. The method for claim 17 wherein the at least one host cell impurity is an endotoxin.

20. The method of claim 17 wherein the hydrophobic interaction media comprises a chromatography support with pendent hydrophobic groups.

21. The method of claim 20 wherein said pendent groups are selected from the group consisting of C₃ to C₁₀ alkyl groups.

22. The method of claim 20 wherein the hydrophobic interaction media is selected from the group consisting of a methacrylate polymer or copolymer backbone bound to a least one of a propyl, butyl, hexyl, octyl, nonyl, or a mixture of these as ligands.

23. The method of claim 20 wherein the media is at least one of a methacrylate ethylene glycol copolymer backbone or a cross-linked agarose.

24. A method of claim 20 wherein the media is a resin in the form of beads in the size range of 15 to 100 μm .

25. The method of claim 17 wherein the salt comprises an anion or cation selected from the group consisting of acetate, phosphate, carbonate, SO_4^{2-} , Cl^- , Br^- , NO_3^- , Mg^{2+} , Li^+ , Na^+ , K^+ and NH_4^+ .

26. The method of claim 25 wherein the salt is ammonium sulfate in a concentration range of 2.5M to 4M.

27. The method of claim 17 wherein the first conditions comprises equilibrating said media with a salt solution containing ammonium sulfate which is present in a concentration range of about 2.5M to 4M.

28. The method of claim 17 wherein the second conditions comprises washing the media with a salt solution containing ammonium sulfate in a concentration of about 2.35M to about 2.45M.

29. The method of claim 17 wherein the said third conditions comprises washing said second bound mixture with a salt solution containing ammonium sulfate in a concentration of about 1M to 2.3M.

30. A method of separating endotoxin from plasmid DNA comprising contacting a mixture of endotoxin and plasmid DNA with a hydrophobic interaction media under conditions where said endotoxin binds said

hydrophobic interaction media to form a complex and separating said plasmid DNA and said complex.

31. The method of claim 30 wherein the salt comprises an anion or cation selected from the group consisting of acetate, phosphate, carbonate, SO_4^{2-} , Cl^- , Br^- , NO_3^- , Mg^{2+} , Li^+ , Na^+ , K^+ and NH_4^+ .

32. The method of claim 30 wherein said mixture further comprises an ammonium salt in a concentration range of 1.5 to 4M.

33. The method of claim 32 wherein said ammonium salt is ammonium sulfate which is present at a concentration of about 2M.

34. The method of claim 30 wherein the hydrophobic interaction media comprises a chromatography support with pendent hydrophobic groups.

35. The method of claim 34 wherein said pendent groups are selected from the group consisting of C_3 to C_{10} alkyl groups.

36. The method of claim 34 wherein the hydrophobic interaction media is selected from the group consisting of a methacrylate polymer or

009250 20582500

copolymer backbone bound to a least one of a propyl, butyl, hexyl, octyl, nonyl, or a mixture of these as ligands.

37. The method of claim 34 wherein the media is at least one of a methacrylate ethylene glycol copolymer backbone or a cross-linked agarose.

38. A method of claim 34 wherein the media is a resin in the form of beads in the size range of 15 to 100 μm .

39. The method of claim 30 wherein said mixture has a pH in the range of about 6.8 to about 7.4.

40. The method of claim 35 wherein the pH is about 7.4

✓ Sub 0913
41. A method of separating supercoiled plasmid DNA from relaxed plasmid DNA comprising contacting a mixture of supercoiled plasmid DNA and relaxed plasmid DNA with a hydrophobic interaction media under a first conditions where both the supercoiled plasmid DNA and the relaxed plasmid DNA bind to said hydrophobic interaction media to form a bound first mixture, altering said first conditions surrounding the bound first mixture to a second conditions to remove said relaxed plasmid DNA from said bound first mixture to form separate components containing a second bound mixture and

009250-052600

said relaxed plasmid DNA, and modifying the second conditions surrounding said second bound mixture to a third conditions to remove said supercoiled plasmid DNA from said second bound mixture to form separate components containing said hydrophobic interaction media and said supercoiled plasmid DNA.

42. The method of claim 41 wherein said hydrophobic interaction media comprises a chromatographic support with pendent hydrophobic groups.

43. The method of claim 42 wherein said pendent hydrophobic groups are selected from the group consisting of C₃ to C₁₀ alkyl groups and mixtures thereof.

44. The method of claim 41 wherein said hydrophobic resin is a methacrylate polymer or copolymer backbone bound to at least one of a propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl , or decyl ligand.

SUB B4) 45. The method of claim 44 wherein said resin is at least one of a methacrylate and ethylene glycol copolymer backbone or a cross-linked agarose.

Sub
art

46. The method of claim 41 wherein said resin is in the form of beads ranging in size from 35 to 100 μm .

47. The method of claim 41 wherein said first conditions comprises equilibrating said mixture and media with a salt solution containing ammonium sulfate in a concentration range of about 2.5 M to about 4 M.

Sub
art
15

48. The method of claim 47 wherein said second conditions comprises washing said first bound mixture with a salt solution containing ammonium sulfate in a concentration of about 2.35 M to about 2.45 M.

49. The method of claim 48 wherein said third conditions comprises washing said second bound mixture with a salt solution containing ammonium sulfate in a concentration of about 1 M to about 2.3M.

50. The method of any one of claims 17 and 41 wherein said altering and said modifying are combined in a continuous process comprising gradient elution of said relaxed plasmid DNA and supercoiled plasmid DNA by mixing said bound first mixture with an ammonium sulfate containing salt solution with a continuously varying concentration of ammonium sulfate, said concentration varying from about 3M to about 1 M ammonium sulfate, and

009250 2052550

said relaxed plasmid DNA is collected in a first eluted volume and said supercoiled plasmid DNA is collected in a second eluted volume.

51. The method of claim 41 wherein said separate relaxed plasmid DNA component and said separate supercoiled plasmid DNA are collected and isolated.

✓ Sub 2/16
09578507-052600

52. A method for the enriching the amount of supercoiled DNA relative to relaxed DNA in a mixture thereof, the method comprising:

- (1) interacting the mixture containing supercoiled DNA and relaxed DNA with a hydrophobic interactive media comprising an alkyl moiety under ionic conditions wherein the supercoiled DNA preferentially binds to the hydrophobic interactive media;
- (2) treating the hydrophobic interactive media containing the relaxed and supercoiled DNA under ionic conditions that allow the preferential removal of the relaxed DNA; and
- (3) eluting the supercoiled DNA from the hydrophobic interactive media

✓ 53. A method for removing lipopolysaccharide (LPS) from a composition containing DNA, the method comprising:

13

- (1) interacting the mixture comprising the DNA and LPS with a hydrophobic interactive media comprising an alkyl moiety, wherein the interacting is under ionic conditions where the LPS preferentially binds to the hydrophobic interactive media relative to the DNA; and
- (2) treating the hydrophobic interactive media containing the DNA and LPS with ionic conditions that allow the selective removal of the DNA.

ADD BS)

009350 0554560